Points to Consider When Applying to the National Institutes of Health Office of Biotechnology Activities (NIH OBA) for a Reduction in Biosafety Level (BL) Containment for Experiments Involving the Cloning of Full-length cDNA Constructs Derived from Risk Group (RG) 4 Viruses of the Order *Mononegavirales*

Background:

RG4 viruses of the order *Mononegavirales* comprise the following two families:

- Ebola and Marburg (Family: *Filoviridae*)
- Nipah and Hendra (Family: *Paramyxoviridae*)

Advances in the knowledge and understanding of the biology of these non-segmented, negative sense, single strand (*ssns*$^{(-)}$) RNA viruses has enabled the construction of complementary DNA (cDNA) molecules of the entire genome of these viruses (termed "reverse genetics") in order to facilitate biological experimentation. Because these RG4 *ssns*$^{(-)}$ RNA viruses do not produce any DNA intermediates throughout their natural life cycle, the construction of cDNA copies of these RNA viral genomes is strictly limited to the laboratory environment and is primarily the result of recombinant DNA techniques. Thus, any cloned cDNA molecule of any RG4 virus of the order *Mononegavirales* and any viral RNA molecules derived from these RG4 viral cDNAs meet the definition of a recombinant DNA molecule as stated in Section I-B of the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines).*[1]

According to Section III-D-2-a of the *NIH Guidelines*, the cloning of full-length genomes of cDNA copies of these RG 4 viruses into non-pathogenic prokaryotes (such as *E. coli* K12) must be performed at BL4 unless a demonstrated "irreversibly defective fraction" of the RG 4 cDNA is used, in which case containment may be reduced to BL2. Because full-length cDNA constructs of the these RG 4 *ssns*$^{(-)}$ RNA viruses can yield infectious forms of virus under specific experimental conditions, and by definition are not fractions of the genome, these cDNA constructs do not meet the criteria stated in the *NIH Guidelines.* Therefore, work with the full-length cDNA in *E. coli* shall be carried out at BL4 under the *NIH Guidelines* unless OBA authorizes the Institutional Biosafety Committee (IBC) to lower containment following an appropriate risk assessment.

Recent OBA Decisions to Lower Containment for work with the full cDNA of Risk Group 4 Mononegavirales in *E. coli*:

The inherent biological characteristics of the full-length genomic cDNA of these viruses in non-pathogenic prokaryotes, such as *E. coli*, led OBA to concur with a recommendation from the Recombinant DNA Advisory Committee (RAC) that lower containment could be appropriate under specific conditions.

The RAC has previously considered requests from Dr. Heinz Feldmann, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases (NIAID), NIH to lower containment for cloning of the full-length cDNA of Ebola, Marburg, Nipah, and Hendra in non-pathogenic *E. coli*. These requests were discussed at the March 2009 and the June 2009 meetings of the RAC. The webcasts and briefing materials are available on the RAC meetings page of the OBA website: http://oba.od.nih.gov/rdna_rac/rac_meetings.html.

In reviewing the **biosafety risks** involved in the cloning of full-length cDNA molecules derived from these RG 4 *ssns*$^{(-)}$ RNA viruses, the RAC concluded that the biological characteristics of these RNA viruses are such that manipulation of full-length viral cDNA in a non-pathogenic prokaryote, such as an *E. coli* K-12 host, can be safely performed under BL2 containment. However, the RAC further acknowledged that biosafety is not the only consideration in allowing such work to proceed at BL2 containment. While the RG4 viruses Ebola, Marburg, Nipah and Hendra, are considered to be Select Agents by the Centers for Disease Control and Prevention (CDC), neither the RNA nor the cDNA of the viruses are currently considered to be Select Agents. However, with proper knowledge, one could deliberately use the full-length cDNA constructs of any of these agents to rescue infectious virus in appropriate mammalian cell lines. Therefore, this work potentially raises **biosecurity** concerns.

The RAC acknowledged that the *NIH Guidelines* primarily address biosafety, but noted that measures taken for biosafety purposes often overlap with those taken for biosecurity purposes. Whereas biosafety focuses on protecting the laboratory worker, public, and environment from inadvertent exposure to, or release of, a microorganism, biosecurity focuses on the "protection of microbial agents from loss, theft, diversion or intentional misuse" (CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories - BMBL* (5th Edition) "Principles of Biosecurity," Section VI). Although there are no specific Federal biosecurity requirements for research with non-Select Agents, such as the cDNA of Ebola, Marburg, Hendra and Nipah, addressing the biosecurity risk of this work is appropriate and is in keeping with the spirit of the *NIH Guidelines*. The *NIH Guidelines* state that "it is the responsibility of the institution and those associated with it to adhere to the intent of the *NIH Guidelines* as well as their specifics." Since the intent of the *NIH Guidelines* is to protect public health, the biosecurity risks of conducting this research at BL2 should also be considered.

With the addition of a stipulation to implement appropriate measures to address potential biosecurity risks, as recommended by the RAC, OBA concurred and authorized the IBC at Rocky Mountain Laboratories, NIAID, NIH to lower containment to BL2 for work with the cDNA of Ebola, Marburg, Nipah and Hendra viruses in non-pathogenic *E. coli*. The approval is specific to Dr. Heinz Feldmann at Rocky Mountain Laboratories.

This document outlines the information that is to be submitted to OBA when requesting OBA's approval to lower containment for work with the full-length cDNA of these RNA viruses, Ebola, Marburg, Hendra and Nipah, in non-pathogenic *E. coli*.

Requirements for Applications to NIH OBA for Lowering of Containment for the Cloning of the Full-length cDNA of RG 4 *ssns*$^{(-)}$ RNA Virus Genomes in Non-Pathogenic Strains of *E. coli*.

Investigators should provide written evidence that appropriate biosafety and biosecurity controls, which are commensurate with the risk of the proposed work, will be implemented at their institution. The application for reduction in biosafety containment should include (1) a description of the biosafety risk assessment undertaken in accordance with the *NIH Guidelines* and (2) a biosecurity assessment and a plan that is consistent with "Principles of Laboratory Biosecurity" outlined in Section VI of the *BMBL* (5th Edition):

- Investigators or institutions that are approved to work with Select Agents are to provide non-sensitive information in the application document in accordance with applicable regulations.
- NIH OBA will review and approve submitted applications on a case-by-case basis. Further evaluation by the RAC may be warranted in certain cases.

The following is a summary of the essential elements that, at minimum, should be included in the application document.

Section 1: Statement of Work and Justification

- State concisely the overall objectives and rationale of the proposed study.
  - Include information to justify the need for cloning full-length cDNA genome copies of RG4 *ssns*$^{(-)}$ RNA virus(es) (*e.g*. that such work is necessary because the investigator intends to use the cDNA genome copies to rescue infectious virus in a BL4 facility).

Section 2: Description of the Biological System(s) and Experimental Manipulations

- Describe all biological reagents (*e.g.* plasmids, cell lines, prokaryotic hosts) that will be used in the experiment.
  - Describe the types of experiments to be performed.
  - Identify all essential biological reagents required for the rescue of infectious recombinant RG 4 virus.
- Describe the types of recombinant DNA manipulations to be performed and the biosafety containment levels at which each of these operations will occur.

Section 3: Biosafety and Biosecurity Procedures

- Provide information about the general physical layout of the laboratory space where experiments will be performed.
  - Indicate the containment level for all areas contained within the laboratory space.
  - Identify which area(s) will be dedicated specifically for experiments with the full-length cDNA clones, *i.e*. the "dedicated experimental area" and include a description of primary containment equipment located within.
  - Indicate the level of access (*e.g*. general, controlled, restricted, or secure) to each laboratory area and the physical or procedural measures used to control the desired level of access when working with the full-length cDNA clones.
  - Describe the physical or procedural measures to ensure that adequate controls exist to restrict access to full length cDNA clones (or other critical biological reagents).
  - Describe how materials will flow into and out of the dedicated experimental area.
  - Describe the methods that will be employed for the decontamination or destruction of discarded and waste reagents exiting the dedicated experimental area.
  - Describe the physical and procedural methods to ensure that biological waste exiting the dedicated experimental area will be controlled, accounted for, and securely decontaminated or destroyed.
  - Describe the procedures that will be used to maintain a system of inventory control over biological reagents created, used, discarded or destroyed.
  - Describe the procedures used to ensure appropriate control over full-length cDNA reagents should these be moved to other laboratory areas that are not adjacent to or contained within the dedicated experimental area, or transported to physically distinct locations.

Section 4: Personnel

- Provide information about the personnel who will be performing the work.
  - Describe the level of expertise, experience and training of personnel who will have access to full-length cDNA reagents (*e.g.* full-length cDNA molecules, plasmid constructs, transformed *E. coli*).
  - Describe the screening policies and procedures used to evaluate individuals who have access to full-length cDNA reagents.
    - For security clearances other than Federal Public Trust Level 5 (Minimum Background Investigation for Federal employees) or a Federal Bureau of Investigation Security Risk Assessment (for individuals involved in Select Agent research), investigators should provide a listing of the information sources used in the preparation of the background investigation process

Section 5: Incident Response, Incident Reporting and Training

- Provide information describing the institutional emergency planning and program management.
  - Describe the institutional policies and procedures responsible for managing:
    - Biosafety incidents (exposures or laboratory acquired infections)
    - Biosecurity incidents (thefts or threats)
    - Hazardous materials incidents (spills or releases)
    - Policies and procedures with outside health authorities
- Describe the institutional policies or procedures involving biosafety and biosecurity training, including the frequency of the training requirements.

Section 6: Compliance Assurance and Documentation Requirements

- An appropriate institutional official must be appointed to oversee the biosafety and biosecurity plan of the institution for all work involving full-length cDNA clones of RG4 viruses. Contact information for this individual should be provided.
- The institutional official will be responsible for periodic re-evaluation of the biosafety and biosecurity plan.
- The institutional official will be responsible for filing an annual report with the IBC. The report will include:
  - Any incidents of containment breaches or laboratory exposures
  - Changes in biosafety of biosecurity procedures
  - Documentation of on-going periodic training of personnel in all applicable procedures
- A copy of the annual report will be provided to NIH OBA for review by the RAC as required.

Submission of relevant information should be made to:

Office of Biotechnology Activities
National Institutes of Health
6705 Rockledge Drive, Suite 750
Bethesda, MD 20892-7985 (20817 for non-USPS mail)
Fax: (301) 496-9839
Email: oba@od.nih.gov

[1] Reference in this document to language contained in the *NIH Guidelines* may be incomplete and is intended for illustrative purposes only. Readers are urged to refer back to the *NIH Guidelines* for the complete text contained in all sections of the *NIH Guidelines* cited herein.